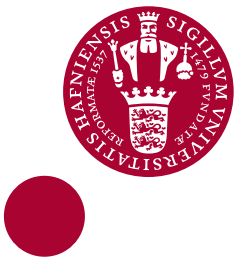

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Published in:
Ecosphere

DOI:
10.1002/ecs2.2290

Publication date:
2018

Document version
Publisher's PDF, also known as Version of record

esa

ECOSPHERE

Soils beneath different arctic shrubs have contrasting responses to a natural gradient in temperature

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Citation: Zhao, Q., M. K. Sundqvist, G. S. Newman, and A. T. Classen. 2018. Soils beneath different arctic shrubs have contrasting responses to a natural gradient in temperature. Ecosphere 9(6):e02290. 10.1002/ecs2.2290

Abstract. Shrubs commonly form islands of fertility and are expanding their distribution and dominance in the arctic due to climate change, yet how soil properties may be influenced when different species of shrubs expand under warmer climates remains less explored. Important plant traits, such as their associated root community, are linked to functionally different and dominant shrub species in the arctic and these traits likely shape biogeochemical cycling in areas of shrub expansion. Using an elevational gradient as a proxy for warming, we explored how biochemical processes beneath two important arctic shrubs varied under warmer (low elevation) and cooler (high elevation) climates. Interestingly, the influence of elevation on biogeochemistry varied between the two shrubs. At the low elevation, *Betula nana* L., an ectomycorrhizal shrub, had high carbon (C) degrading enzyme activities, and relatively low potential net nitrogen (N) mineralization rates. Conversely, *Empetrum nigrum* ssp. *hermaphroditum* Hagerup, an ericoid mycorrhizal dwarf-shrub, had higher enzyme activities and net N immobilization rates at the higher elevation. Further, *E. nigrum* ssp. *hermpahroditum* appeared to have a more closed C and nutrient cycle than *B. nana*—enzymes degrading C, N, and phosphorus were tightly correlated with each other and with total C and ammonium concentrations in the humus beneath *E. nigrum* ssp. *hermaphroditum*, but not beneath *B. nana*. Our results suggest differences in the warming responses of C and N cycling beneath shrub species across an arctic tundra landscape.

Key words: biochemical processes; *ectomycorrhizae; ericoid mycorrhizae;* global warming; shrub species.

Received 22 April 2018; accepted 2 May 2018. Corresponding Editor: Debra P. C. Peters.

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Introduction

Arctic ecosystems are temperature sensitive, have large soil carbon (C) pools, and they will likely respond strongly to climatic warming (Chapin et al. 1995, Tarnocai et al. 2009). Many arctic tundra ecosystems have shallow mineral soils covered by a layer of mor humus where the majority of plant–soil feedbacks occur (Ponge 2013), and where the majority of soil organic C, organically bound nutrients, and plant fine roots are distributed (Handley 1954). Therefore, the biogeochemistry of arctic tundra humus soil plays a pronounced role in tundra nutrient and C dynamics. Further, elevated temperature often promotes soil microbial activity leading to accelerated soil organic matter mineralization and C loss in tundra ecosystems (Nadelhoffer et al. 1992, Rustad et al. 2001, Lavoie et al. 2011). However, soil responses to warming should be linked, at least locally, to the plant community that is present at the site (Kardol et al. 2010, Classen et al. 2015).

Specifically, in tundra ecosystems, large differences in organic soil nitrogen (N) dynamics vary over small spatial scales, where lower net N mineralization rates occur in organic soils beneath heath communities dominated by shrubs and dwarf-shrubs than in soils beneath meadow plant communities (Björk et al. 2007). Individual plant species also exert a strong influence over soil processes forming islands of fertility that influence what plants are able to recruit into an ecosystem (Schlesinger et al. 1990, Berendse 1994, Van Breemen and Finzi 1998, Jiang et al. 2016). One striking response to warming in the arctic is the increased growth and cover of shrubs (Tape et al. 2006, Hudson and Henry 2009), and different species and functional groups of shrubs exhibit strong regional variation in their response to warming in arctic tundra (Wilson and Nilsson 2009, Myers-Smith et al. 2011, Elmendorf et al. 2012). The impact of shrub expansion may be contingent on the traits and characteristics of the expanding shrub. Thus, an understanding of how functionally different shrubs influence soil properties is important if we are to predict the soil function, as well as the plant composition, of arctic ecosystems in the future.

Some plants have traits that strongly influence the way they obtain and cycle nutrients within their sphere of influence. Aboveground, plant leaf and litter traits impact litter decomposability and the amount of nutrients that are released to the soil during decomposition (Berendse 1994, Cornwell et al. 2008). Belowground, a plant trait that has a strong impact on nutrient cycling is its mycorrhizal associate. In fact, classifying plants by their mycorrhizal associations predicts many interspecific plant C and nutrient cycling traits, such as litter decomposability and soil C storage (Cornelissen et al. 2001, Averill et al. 2014). In arctic tundra, most shrubs are associated with ectomycorrhizal (ECM) fungi (i.e., *Betula* and *Salix*) or ericoid mycorrhizal (ERM) fungi (dwarf-shrubs in *Ericaceae,* i.e., *Empetrum* and *Vaccinium*); two fungal types that have contrasting influences on soil properties (Read et al. 2004, Parker et al. 2015). Many ERM shrubs produce litter rich in polyphenolic compounds, and ERM fungi correspondingly have a stronger ability to depolymerize protein–tannin complexes than ECM fungi (Bending and Read 1997, Tybirk et al. 2000). There is evidence that phenolic-rich litter from ERM shrubs have strong adverse effects on C and nutrient mineralization (Castells et al. 2005, González et al. 2015). While field studies at the community level show variation in soil C stocks, mineralization and respiration rates under different vegetation types where both ECM and ERM shrubs are present (Chu and Grogan 2010, Parker et al. 2015), a direct comparison of soil functioning between shrubs with these contrasting traits under naturally varying climatic conditions across the arctic landscape is limited.

Plant–soil feedback theory suggests that, under changing environments, plants can regulate the magnitude of their impacts on soil processes to reinforce their fitness in new environments (Berendse 1994, Jeffers et al. 2012, Miki 2012). So, plant species with different nutrient economies may regulate their impacts on soil processes in different ways. To date, there is a large variation in the responses of plant leaf and litter traits to changes in environmental conditions, including warming, both among species and study systems (e.g., Graglia et al. 2001, Kaarlejärvi et al. 2012, De Long et al. 2016). Similarly, ECM fungal biomass generally responds positively to warming, while ERM fungal biomass responses are variable suggesting that fungal associates also have a wide range of responses to changes in the environment (Olsrud et al. 2004, Clemmensen et al. 2006).

Extracellular enzymes produced by fungi and plant roots play a key role in regulating soil C and nutrient transformations in arctic tundra (Burns and Dick 2002, Tarnocai et al. 2009). Previous studies suggest that extracellular activities in arctic ecosystems are not only constrained by low temperatures, but also by low concentrations of available N, accessibility of organic C, and soil pH (Wallenstein et al. 2009, Stark et al. 2014, Melle et al. 2015). For example, as N constitutes an important component of microbial and enzymatic makeup, synthesis of extracellular enzymes can be limited by the deficiency of inorganic N (Sinsabaugh et al. 2008, Sistla et al. 2012, Stark et al. 2014). In addition, soil extracellular enzymes degrading C, N, and phosphorus (P) can be limited by different factors, and thus respond differently to environmental change. Generally, C-degrading enzymes respond positively to N and labile C addition, while responses of N- and P-degrading enzymes can vary greatly to changes in C (Stark et al. 2014, Melle et al.

2015). While plants and their associated traits influence soil properties in arctic ecosystems, little is known on how different plant species influence the amount of extracellular enzymes produced by microbes in the soils beneath them, how these patterns are linked with soil processes and interacts with environmental conditions that vary across a tundra landscape.

Here, we investigated whether the responses of belowground biochemical processes to warming in arctic tundra differed between two arctic shrubs with distinct traits (ECM vs. ERM). We used an elevational gradient in northern Sweden (e.g., Sundqvist et al. 2011, Vincent et al. 2014, De Long et al. 2015) to explore the effect of elevation-associated changes in temperature on belowground processes for two common species of arctic shrubs that have contrasting traits, the evergreen dwarf-shrub *Empetrum nigrum* ssp. *hermaphroditum* Hagerup. and the deciduous shrub *Betula nana* L. Both of these species have responded positively to global warming in different locations and thus are likely to expand their range in the future (Chapin et al. 1995, Wilson and Nilsson 2009, Myers-Smith et al. 2011). *B. nana* is obligately symbiotic with ECM fungi, while *E. nigrum* ssp. *hermaphroditum* associates with ERM fungi. We compared soil enzyme activities and other soil biotic and abiotic variables related to C, N, and P cycling in the humus layer beneath each species at two elevations (a site located at 500 m and a site located at 900 m). We hypothesized that (1) the humus layer beneath *E. nigrum* ssp. *hermaphroditum* would be deeper and associated with lower nutrient availability and microbial activity than in humus beneath *B. nana,* because litter of *E. nigrum* ssp. *hermaphroditum* is lower in nutrients than that of *B. nana* and it contains an allelopathic compound (batatasin-III) that can depress soil biological activity (Castells et al. 2005, González et al. 2015); (2) soil biochemical processes beneath *E. nigrum* ssp. *hermaphroditum* and *B. nana* would vary with elevation in contrasting ways in our study system given their contrasting associated above- and belowground traits (Berendse 1994). Specifically, *B. nana* would have relatively high rates of soil C and nutrient transformations at the warmer, low-elevation site in our study system compared to *E. nigrum* ssp. *hermaphroditum* because ECM colonization facilitates C turnover, and because ECM colonization rates can increase in *B. nana* under warming (Clemmensen et al. 2006, Deslippe and Simard 2011). In contrast, ERM-associated *E. nigrum* ssp. *hermaphroditum* would slow soil C and nutrient transformations and these impacts will counteract, or even be larger than, the direct effects of elevated temperature. Hence, we hypothesized that in our study system C and nutrient transformations in soils beneath *E. nigrum* ssp. *hermaphroditum* would be less responsive to elevation-associated differences in temperature than in soils beneath *B. nana*.

Materials and Methods

Site description

We conducted this study at 500 m and 900 m situated along an elevation gradient on the northeast facing side of Mt Suorooaivi (1193 m a.s.l.), located 20 km southeast of Abisko, northernmost part of Sweden (68°21′ N, 18°49′ E; e.g., Sundqvist et al. 2011, Vincent et al. 2014, De Long et al. 2015). The bedrock consists of salic igneous rocks and quartic and phyllitic hard schists (SGU 1965). The climate in this area is subarctic and the growing season lasts approximately three months. The mean annual precipitation in Abisko (1913–2000) was 310 mm with the highest mean monthly precipitation occurring in July (51 mm) and the lowest precipitation occurring in April (12 mm; Kohler et al. 2006); precipitation varies little among the elevations measured in this study (Karlsson et al. 2005). During the summer months, air and soil temperature declines with elevation across this study system (Sundqvist et al. 2011, Graae et al. 2012); air temperature is approximately 2.0–2.5°C higher at the 500 m site than at the 900 m site (Blüme-Werry et al. 2017). The heath vegetation at the sites is dominated by ericaceous dwarf-shrubs, predominantly the evergreen dwarf-shrub *Empetrum nigrum* ssp. *hermaphroditum,* as well as the deciduous shrub *Betula nana*. We sampled plant cover in July 2014 and we found that *E. nigrum* ssp. *hermaphroditum* made up 59% and 73% of total community cover at 900 m and at 500 m, respectively. The cover of *B. nana* was 35% and 32% at 900 m and at 500 m, respectively (*unpublished data*). Other ericaceous dwarf-shrubs in these communities are *Vaccinium vitis-idaea*, with a cover of 5% and 4% at the 900 and 500 m, and *Vaccinium uliginosum*, with a

cover of 1% and 4% at the 900 and 500 m, respectively (*unpublished data*). *Arcthostaphylos alpinus* also occurs, but with <1% cover at both elevations, and at the high elevation, the graminoids *Calamagrostis lapponica* and *Carex bigelowii* occur with a cover of 3% and 2%, respectively (*unpublished data*).

Soil sampling

On 8 July 2015, we collected the humus layers beneath *B. nana* and beneath *E. nigrum* ssp. *hermaphroditum* at each of the two elevations. For each species at each elevation, 10 plots (1 × 1 m) were randomly selected of similar slope and aspect within the heath vegetation where the cover of each species was ≥80% in each plot, with a minimum distance of 10 m and a maximum distance of 50 m for spatial independence among soil samples (Baldrian 2014). Three cores (8 cm diameter, 10 cm deep) were collected immediately beneath each species within each plot and homogenized into a single sample, rendering 10 samples for *B. nana* and 10 samples for *E. nigrum* ssp. *hermaphroditum* for each elevation. The depth of the humus layer was recorded for each soil core. Samples were brought back to the laboratory and stored at 4°C until further analysis. We homogenized and sieved (4 mm mesh) each of the collected samples and then analyzed each sample for gravimetric moisture content, pH, available N and P, potential net N mineralization, and enzyme activities. A subsample from each sample was dried at 105°C and ground to a fine powder to measure total organic C, total N, and total P.

Soil physicochemical analyses

We measured gravimetric soil moisture by drying samples at 105°C to a constant weight. We determined pH by suspending 5 g of field-moist humus in 30 mL of DI water (v/v ratio is about 1:2) and then measuring the suspension with a pH meter (Kalra 1995). Total C and N content were determined by dry combustion and non-dispersive infrared absorption analysis (LECO Corporation, St. Joseph, Michigan, USA). Total P was determined by H_2SO_4-Se digestion and colorimetric analysis. Available P was determined using Bray-1 extraction method (0.03 mol/L NH4F + 0.025 mol/L HCl) with 1:10 humus to solution ratio (Bray and Kurtz 1945), and phosphate in the extracts was measured colorimetrically based on the reaction with ammonium molybdate on a microplate reader (Kuo 1996). Mineral N (NH_4^+−N and NO_3^-−N) were extracted with 2 mol/L KCl solution and determined colorimetrically on a microplate reader based on the methods of Doane and Horwath (2003) and Weatherburn (1967). To measure potential net N mineralization, 10 g of field-moist humus sample was incubated in a cup that was placed in a 1-L jar for 14 d at room temperature (20° ± 2°C) in dark, and the moisture was kept constant by adding 5 mL distilled water in the jar to prevent water loss. Concentrations of NO_3^-−N were very low (<4 mg/kg) and even below detection limit in 30% of the samples. The difference in inorganic N (the sum of NH_4^+−N and NO_3^-−N) pools after and before incubation was used to estimate the rates of potential net N mineralization over the incubation period.

Enzyme assays

We measured the potential activities of six soil hydrolytic enzymes: acid phosphatase (AP), N-acetylglucosaminidase (NAG), β-glucosidase (BG), α-glucosidase (AG), Cellobiohydrolase (CB), and β-xylosidase (BX), of which AP is the key enzyme of soil organic P mineralization, NAG is the N acquiring enzyme, and the other four enzymes are key in degrading organic C. Specifically, AP hydrolyzes phosphate esters to soluble phosphate, NAG releases N-acetyl glucosaminide from chitin-derived oligomers, BG releases glucose from cellulose, AG releases glucose from soluble saccharides, CB releases disaccharides from cellulose, and BX degrades hemi-cellulose (Sinsabaugh et al. 2008). Enzyme activities were assayed according to the high-throughput fluorometric method in 96-well microplates described by Bell et al. (2013), using fluorescent labeled 4-methylumbelliferone (MUB)-linked substrates. Briefly, two deep well plates were prepared, one MUB standard plate for building the standard curve of fluorescence intensity of each sample, and one sample plate for measuring the fluorescence intensity of the sample. The slurry of the humus was prepared by homogenizing 1.5 g of field-moist humus with 80 mL of sodium acetate buffer solution (pH 5.0) in a blender. Then, a volume of 800 μL of humus slurry was pipetted into wells of the standard plate with 200 μL of MUB standard solution and

wells of the empty sample plate, respectively. This step was followed by adding 200 μL of the substrate solution to wells of the sample plate, after which both standard and sample plates were homogenized thoroughly on a shaker for 2 min. The plates for phosphatase were then incubated for 1 h, and plates for all other enzymes were incubated for 3 h at room temperature (20° ± 2°C). Fluorescence was measured on a microplate reader with excitation at 365 nm and emission at 460 nm. Enzyme activities were expressed as nmol of substrate converted per gram of dry soil per hour (nmol $g{\cdot}soil^{-1}{\cdot}h^{-1}$).

Statistical analyses

Two-way analyses of variance (ANOVA) were used to test for the main and interactive effects of elevation and species on soil biochemical properties. Whenever a significant elevation × species interaction was found, one-way ANOVA was used to test the effect of elevation on each species separately. All data were tested for assumptions of normality and homogeneity of variance prior to ANOVA and were natural logarithmically (ln) transformed to satisfy these assumptions when required. Statistically significant differences were accepted at $P \leq 0.05$. ANOVA analyses were performed in SPSS statistical software package version 11.5 (SPSS Inc., Chicago, Illinois, USA).

To explore the links among soil characteristics and enzyme activities, a constrained redundancy analysis (RDA) was performed using six enzyme activities as response variables and using all soil physiochemical properties as explanatory environmental variables, where each explanatory variable that contributed significantly to the explained variation at $P < 0.05$ (determined using Monte Carlo permutation tests with 999 permutations) was retained by forward selection (ter Braak and Šmilauer 2002). Next, we built a structural equation model (SEM) to quantitatively explore the main soil characteristics and pathways through which they might influence enzyme activities. Structural equation model is a statistical technique for testing causal relations using a combination of statistical data and qualitative causal assumptions (Grace 2006). Based on results of RDA analyses as well as general theory of enzyme activity and published studies on the interacting effects of soil characteristics on enzyme activities in tundra regions (Sinsabaugh et al. 2008, Wallenstein et al. 2009, Stark et al. 2014, Melle et al. 2015), we constructed a priori model with soil physiochemical properties significantly impacting enzyme activities directly as predictors and with enzymes acquiring C, N, and P (E_C, E_N, and E_P) as endogenous variables. More specifically, substrate availability, nutrient availability, and pH were the main factors influencing enzyme activity (Sinsabaugh et al. 2008). In (sub-)arctic tundra ecosystems, N-limitation as well as low soil pH, available N, and organic C are among the main factors influencing extracellular enzyme activity (Wallenstein et al. 2009, Stark et al. 2014, Melle et al. 2015). In addition, our RDA analyses showed that NH_4^+−N, total C, total N, and total P and pH are the main soil physiochemical properties influencing enzyme activities at our study site.

Based on previous findings (Wallenstein et al. 2009, Stark et al. 2014, Melle et al. 2015) and our results from the RDA following removal of highly correlated environmental variables (total N and total P were removed as they were highly correlated with total C), we included pH, total C, and NH_4^+−N as predictors in our *priory* model. The E_C used in the SEM was calculated as the sum of BG, AG, CB, and BX (Bell et al. 2013). We performed model estimation using maximum likelihood procedures. We based model fit on chi-square values (χ^2) and their associated *P*-values and judged a model as fitting the structure when $P > 0.05$. The goodness of fit and root square mean error of approximation were also used to assess the fitness of the model. Based on the results of goodness-of-fit tests, non-significant paths were removed stepwise from the model, and the final models that best fit the data were obtained (Arbuckle 2011). All multivariate analyses were conducted separately for *E. nigrum* ssp. *hermaphroditum* and *B. nana*, and data were ln $(X + 1)$ transformed prior to analyses. Redundancy analysis was performed on CANOCO 4.5, and SEM was operated on AMOS 20 software (SPSS Inc., Chicago, Illinois, USA).

Results

Soil enzyme activities

Elevation interacted with species to influence soil enzyme activity for each of the enzymes we measured with the exception of NAG activity (Table 1). These interactions occurred because

Table 1. Results from two-way ANOVA (*F* and *P* values) on the main effects of elevation (500 m vs. 900 m), shrub species (*E. nigrum* ssp. *Hermaphroditum* vs. *B. nana*), and their interactions on soil enzyme activities and physiochemical properties.

	Elevation (E)		Species (S)		E × S interaction	
Variables	*F*	*P*	*F*	*P*	*F*	*P*
BG	0.07	0.796	3.17	0.084	12.20	**0.001**
AG	0.50	0.483	5.86	**0.021**	24.38	**0.000**
CB	3.59	0.066	1.78	0.191	7.80	**0.008**
BX	58.89	**0.000**	2.57	0.118	5.10	**0.030**
NAG	0.04	0.850	2.07	0.159	3.27	0.079
AP	64.73	**0.000**	7.50	**0.010**	13.01	**0.001**
pH	31.89	**0.000**	0.04	0.845	0.13	0.726
Total C	1.94	0.173	1.28	0.266	3.70	0.062
Total N	8.45	**0.006**	17.33	**0.000**	3.48	0.070
Total P	94.50	**0.000**	3.93	0.055	1.80	0.188
C/N ratio	4.94	**0.033**	17.73	**0.000**	0.19	0.733
Bray-1 P	0.44	0.511	14.81	**0.000**	0.91	0.346
NH_4^+–N	5.81	**0.021**	16.31	**0.000**	0.09	0.763
N mineralization	7.79	**0.008**	13.34	**0.001**	11.34	**0.002**

Notes: Significant *P* values ($P < 0.05$) are highlighted in bold. BG, β-glucosidase; AG, α-glucosidase; CB, Cellobiohydrolase; BX, β-xylosidase; NAG, N-acetylglucosaminidase; AP, Acid phosphatase.

BG and AG concentrations were significantly higher at the high-elevation site than at the low site for humus beneath *Empetrum nigrum* ssp. *hermaphroditum*, while the reverse pattern was found for *Betula nana* (Fig. 1). Further, CB concentrations were significantly higher at the high compared to at the low-elevation site in humus under *E. nigrum* ssp. *hermaphroditum*, while CB concentrations did not differ between elevations for *B. nana* (Fig. 1). Concentrations of BX and AP were both significantly higher at the high-elevation than the low-elevation site in humus under both species, and these differences between elevations were greatest for *E. nigrum* ssp. *hermaphroditum* (Fig. 1). Species identity also directly impacted enzyme activities for AG and AP (Table 1). Overall, the humus beneath *E. nigrum* ssp. *hermaphroditum* had a 24% higher AP and 18% lower AG activities than the humus beneath *B. nana*.

Soil physicochemical properties

We found no significant interaction between elevation and species on humus total C, N, and P concentrations, the C: N ratio, or pH (Table 1). Concentrations of total C and N in the humus beneath *E. nigrum* ssp. *hermaphroditum* were significantly lower at the low-elevation than at the high-elevation site; but, concentrations of C and N did not vary significantly in the humus beneath *B. nana* between low and high sites (Table 2). Total P concentrations were significantly (38–52%) lower at the low-elevation than at the high-elevation site for both shrub species. The ratio of C: N did not change with elevation beneath either species (Table 2). Species had significant direct effects on total N and the C: N ratio but not on total C and P (Table 1). Total N concentrations were 25% lower in the humus beneath *E. nigrum* ssp. *hermaphroditum* than beneath *B. nana*. Soil pH was only affected by elevation, where pH was higher at the low-elevation site (Table 2). The humus layer was 65% thicker beneath *E. nigrum* ssp. *hermaphroditum* than beneath *B. nana*, and depth did not change with elevation for *E. nigrum* ssp. *Hermaphroditum*, but humus depth was 35% lower beneath *B. nana* at the low-elevation than at the high-elevation site (Table 2).

Bray-1 P concentrations were not affected by elevation for either shrub species. Soil NH_4^+–N concentrations tended to be lower at the low-elevation site for both species, but the difference between elevations was only significant for *E. nigrum* ssp. *hermaphroditum* (Table 2). Species and elevation directly and interactively impacted potential net N mineralization rates (Table 1). Potential N immobilization occurred at both

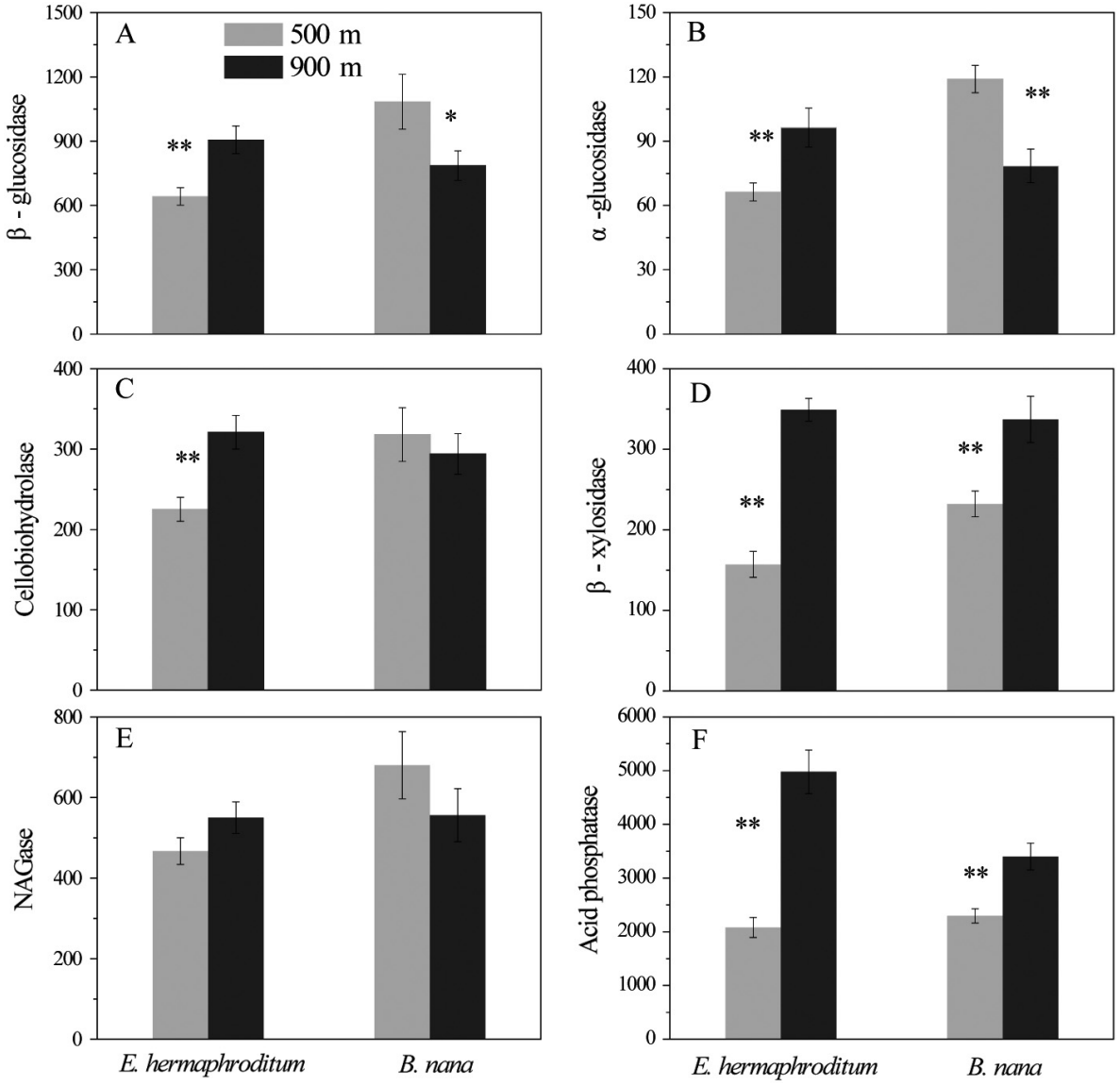

Fig. 1. Enzyme activities (nmol g·soil^{-1}·h^{-1}) in the humus beneath *E. nigrum* ssp. *hermaphroditum* and *B. nana* at a low elevation and a high elevation (Mean with standard error, $n = 10$). (A) β-glucosidase; (B) α-glucosidase; (C) Cellobiohydrolase; (D) β-xylosidase; (E) NAGase; (F) Acid phosphatase. ** and * denotes significant elevation effect at $P < 0.01$ and $P < 0.05$, respectively.

elevations and was greatest at the high-elevation site beneath *E. nigrum* ssp. *Hermaphroditum*. Contrastingly, N immobilization occurred at the low and N mineralization occurred at the high-elevation site beneath *B. nana*. Species had significant direct effects on Bray-1 P, NH_4^+−N concentrations, and N mineralization rate, with these variables being lower beneath *E. nigrum* ssp. *hermaphroditum* than *B. nana* (Tables 1 and 2).

Relationship between soil enzyme activities and physicochemical properties

The RDAs revealed that enzyme activities were correlated with several soil physiochemical properties, but these patterns strongly differed by shrub species (Fig. 2). For *E. nigrum* ssp. *hermaphroditum,* axis 1 explained 85.6% of the relationship among soil enzyme activities and soil physiochemical variables. In fact, all of the

Table 2. Physiochemical properties of the humus beneath *E. nigrum* ssp. *hermaphroditum* and *B. nana* collected at a low and a high elevation.

Plant species	Elevation (m)	Humus depth (cm)	pH	Total C (g/kg)	Total N (g/kg)	Total P (g/kg)	C:N	Bray1-P (mg/kg)	NH_4^+−N (mg/kg)	Net N min. (mg/kg 14 d^{-1})
E. nigrum ssp. *hermaphroditum*	500	6.59 (1.03)	4.56 (0.05)	365.9 (37.4)	9.32 (0.94)	0.78 (0.04)	39.3 (0.71)	39.9 (6.15)	3.81 (0.34)	−0.42 (0.13)
	900	6.65 (0.43)	5.11 (0.11)	474.0 (30.7)	13.7 (0.55)	1.62 (0.12)	34.9 (2.65)	38.1 (4.66)	7.60 (0.28)	−1.09 (0.29)
P value		0.958	**0.000**	**0.038**	**0.001**	**0.000**	0.150	0.816	**0.000**	**0.045**
B. nana	500	3.23 (0.30)	4.51 (0.03)	465.6 (29.3)	14.8 (1.06)	1.03 (0.04)	31.5 (1.11)	58.0 (6.28)	9.88 (1.02)	−0.08 (0.20)
	900	4.85 (0.32)	5.12 (0.17)	448.1 (32.5)	15.8 (1.03)	1.67 (0.08)	28.6 (1.51)	68.2 (7.64)	12.8 (2.56)	7.19 (2.33)
P value		**0.002**	**0.006**	0.696	0.526	**0.000**	0.113	0.318	0.300	**0.006**

Notes: Means with one standard error in parentheses, $n = 10$. *P* values are calculated from one-way ANOVA of the effects of elevation on soil variables for each shrub species separately. Significant *P* values ($P < 0.05$) are highlighted in bold. N min., potential net N mineralization rate.

soil physiochemical variables, except for Bray-1 P and C: N ratio, significantly contributed to and were highly correlated with axis 1 ($|r| = 0.519$–0.936, $P < 0.05$, Fig. 2A). According to the Monte Carlo permutation test with 999 unrestricted permutations, the contribution to the variance of enzyme activities was greatest for NH_4^+−N, total C, and N ($P \leq 0.001$), medium for pH, total P ($P < 0.01$), and lowest for N mineralization ($P = 0.024$). In contrast, for *B. nana*, the axis 1 and axis 2 explained 54.1% and 30.1% of the relationships among enzyme activities and soil physiochemical variables, respectively. Total C, total P, and pH contributed significantly to explain the variance of enzyme activities among our samples ($P < 0.05$, Monte Carlo permutation tests; Fig. 2B). Axis 1 represented the variance of enzymes acquiring C and N, and it was significantly correlated with total soil C and pH ($r = -0.572$, $P = 0.008$, and $r = 0.529$, $P = 0.016$). Axis 2 represented the variance of BX and AP activities, and it was correlated with total P ($r = -0.559$, $P = 0.010$, Fig. 2B).

We fitted significant SEMs describing the paths where soil physiochemical properties affected enzyme activities for both species, but the model was a much better fit for *E. nigrum* ssp. *hermaphroditum* ($\chi^2 = 3.538$, df = 5, $P = 0.618$) than for *B. nana* ($\chi^2 = 12.180$, df = 8, $P = 0.143$, Fig. 3). The SEM model explained 84.7%, 47.4%, and 85.3% of the variances of the enzymes acquiring C, N, and P (E_C, E_N, and E_P) for *E. nigrum* ssp. *hermaphroditum*, but explained only 25.1%, 31.7%, and 52.5% of them for *B. nana*. For *E. nigrum* ssp. *hermaphroditum*, E_C and E_N were strongly impacted by total C and pH, but not NH_4^+−N. E_P was strongly impacted by pH, NH_4^+−N, and total C (Fig. 3A). For *B. nana*, E_C was significantly impacted by total C, and E_N was impacted by NH_4^+−N and pH, while E_P was only impacted by pH (Fig. 3B).

Discussion

Species-specific soil responses to elevational gradients of temperature

Plants impact the soils beneath them, impacts that can influence interactions as well as the plant species that are able to establish and expand into those areas the future (Berendse 1994, Van Breemen and Finzi 1998, Ehrenfeld et al. 2005, Jiang et al. 2017). As shrubs expand in the arctic due to climatic warming, they will expand their influence on the soils they grow in and lead to feedbacks that will shape how these ecosystems respond to and develop under a warmer and more variable world (e.g., Buckeridge et al. 2010). In line with our first hypothesis, we found species-specific effects on belowground biochemical processes where humus beneath *Betula nana* had more total N, available N and P, and higher net N mineralization rates than soil beneath *Empetrum nigrum* ssp. *hermaphroditum* (Table 2). Also, some soil enzyme activities (α-glucosidase and acid phosphatase) differed significantly between two shrub species (Fig. 1), suggesting different

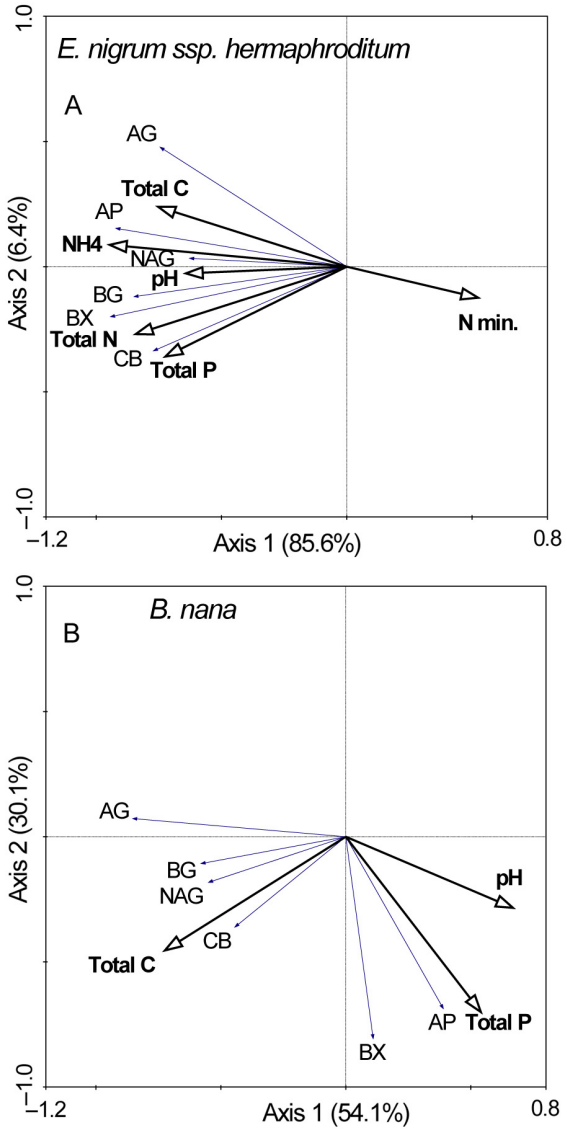

Fig. 2. Ordination diagrams from constrained redundancy analysis (RDA) of the relationship between soil physiochemical variables and enzyme activities for *E. nigrum* ssp. *hermaphroditum* (A) and *B. nana* (B). The relationship between physiochemical variables and enzyme activities explained 92.0% of the variance of enzyme activities for *E. nigrum* ssp. *hermaphroditum*, and 84.2% for *B. nana*. The eigenvalues for the first and second axis were 0.761 and 0.057 for *E. nigrum* ssp. *hermaphroditum*, and 0.296 and 0.164 for *B. nana*.

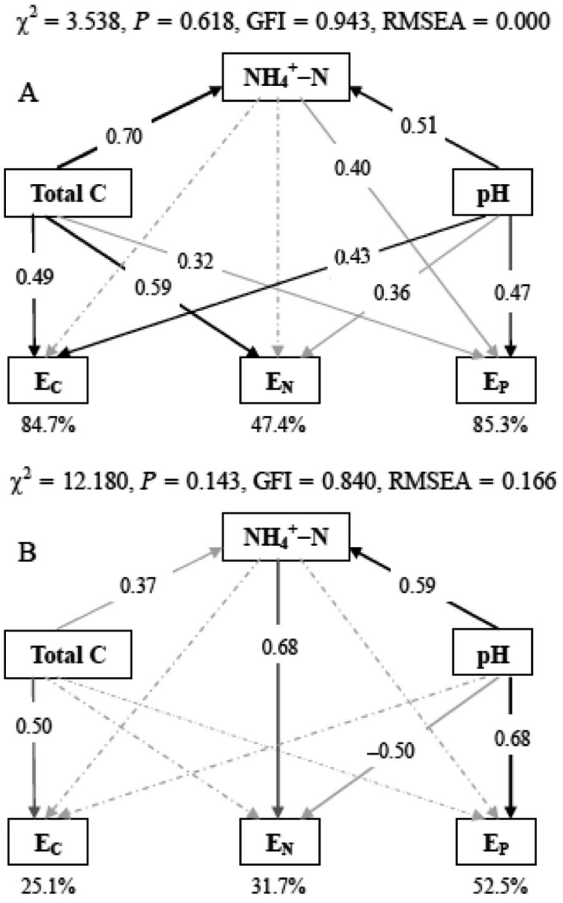

Fig. 3. Structural equation models (SEM) of physiochemical properties as predicators of enzymes acquiring C, N, and P in the humus beneath *E. nigrum* ssp. *hermaphroditum* (A) and *B. nana* (B). Pathways are accompanied by standardized regression coefficients that indicate the strength of the effects. Dotted gray lines indicate non-significant paths ($P > 0.05$) or paths removed to improve fits. Solid lines with different colors denote paths with different significant levels (medium gray indicates $P \leq 0.05$; dark gray indicates $P \leq 0.01$; and black indicates $P \leq 0.001$). Percentages close to endogenous variables indicate the variances explained by the model. NH_4^+–N, ammonium concentration; total C, total carbon concentration; E_C, the sum of all C-degrading enzyme activities; E_N, N-acetylglucosaminidase activity; E_P, acid phosphatase activity.

abilities of ERM and ECM shrubs associated soil microorganisms to degrade organic C and scavenge for organic sources of P. Taken together, these results provide support for how functional differences among plant species are important drivers of many soil biochemical processes (Hobbie 1992, Wardle et al. 2004, Kardol et al. 2010, Classen et al. 2015).

We hypothesized that belowground biochemical processes beneath *E. nigrum* ssp. *hermaphroditum* and *B. nana* would vary in different directions with elevation. Partly consistent with this hypothesis, activities for enzymes degrading labile organic matter (β-glucosidase, α-glucosidase, and cellobiohydrolase) were highest beneath *B. nana* at the lower and warmer elevation site. However, we observed the opposite pattern in humus beneath *E. nigrum* ssp. *hermaphroditum* (Fig. 1). In contrast to soil C degradation, the activity of nutrient acquiring enzymes did not show opposite patterns with species and elevation (Fig. 1). For acid phosphatase, a P acquiring enzyme, enzyme activities for both species were higher at the high-elevation relative to the low-elevation site, but the difference was much greater in soils beneath *E. nigrum* ssp. *hermaphroditum* than *B. nana*. Further, there was no detectable difference in NAG activity, an enzyme mineralizing organic N, at either elevation or beneath either species. Potential N mineralization was negative (i.e., net N immobilization) in most samples and varied in opposite directions with elevation for the two shrub species we studied. Specifically, net N mineralization decreased from the high to low site for *B. nana*, probably because of greater microbial immobilization at the lower and warmer elevation, while net N immobilization was highest at the high site for *E. nigrum* ssp. *hermaphroditum.* Taken together, these results may point to differences in the amounts of soil labile C available for microbial mineralization in humus under these two species across our study system. They are in line with observations of low or negative net N mineralization rates during the growing season in arctic soils, linked with low nutrient availability and microbial immobilization (Schmidt et al. 2002, Chu and Grogan 2010).

Somewhat surprisingly, the response of nutrient availability in the humus beneath each species and at the two elevations varied for N and P. Available P was similar between the high and the low site as well as in soils beneath the two shrub species. However, the concentration of available NH_4^+−N in soils increased significantly with elevation. While the mechanisms behind our results remain inconclusive, we find both elevation and shrub species to affect belowground C, N, and P transformations and nutrient availability. Our results reflect the important role of shrub species in the responses of soil biochemistry to warming in these arctic ecosystems. While elevated temperature can directly promote litter decomposition and microbial activity, and thus increase inorganic nutrient release (Hobbie 1996, Rustad et al. 2001), our results show that elevation, used as a proxy for long-term warming, interacts with dominant arctic shrubs to influence soil processes, in particular C and N turnover—patterns that will shape Arctic ecosystem responses over time.

While much research demonstrates that ECM shrubs have expanded in arctic tundra, increased growth and expansion of ericaceous shrubs, such as *E. nigrum* ssp. *hermaphroditum,* have been observed in long-term warming experiments and observational studies (Wilson and Nilsson 2009, Kaarlejärvi et al. 2012, Zamin et al. 2014). The somewhat contrasting patterns of belowground processes for *E. nigrum* ssp. *hermaphroditum* and *B. nana* across a colder and a warmer elevation may indicate that expansion of either of these two species would cause contrasting feedbacks to climate change. Arctic vegetation dominated by *B. nana* can have high respiration and C turnover rates (Parker et al. 2015). Our findings of greater labile C degradation enzyme activities under *B. nana* at a lower warmer elevation show that elevated temperatures further accelerate C degradation, which could potentially increase C loss beneath *B. nana*. In contrast, our findings suggest that an expansion of *E. nigrum* ssp. *hermaphroditum* under warmer climate would decrease the C loss.

Coupling of C, N, and P cycles

In arctic tundra ecosystems, C and nutrient cycling are often tightly coupled through vegetation and microbial processes because nutrients are limiting to both plants and soil communities, and plant available nutrients are released via mineralization of organic matter (Jonasson et al. 1999, Hobbie et al. 2002, Jiang et al. 2016). Using RDA and SEM analyses, we found large differences in the coupling between C turnover and nutrient mineralization, as well as in the factors influencing mineralization, in soil beneath two functionally distinct shrub species (Figs. 2, 3). Belowground C, N, and P transformations were tightly coupled beneath *E. nigrum* ssp. *hermaphroditum* as indicated by the correlations between enzymes degrading C, N, and P compounds, while beneath *B. nana*

enzymes degrading soil C and N were closely coupled with each other, but not with the enzyme degrading organic P. Organic P is mainly ester-bonded in soils and can be mineralized independently of C mineralization, while N mineralization is not, thus leading to the uncoupling of P mineralizing enzyme from C and N mineralizing enzymes (McGill and Cole 1981).

It is well recognized that microbial biomass and activity can be significantly limited by N deficiency in arctic tundra (Hobbie et al. 2002, Sistla et al. 2012). Low soil pH and low labile soil C can also limit microbial biomass, respiration, and enzyme activities independently and jointly with available N (Whittinghill and Hobbie 2012, Stark et al. 2014, Melle et al. 2015). In the present study, more than 80% of the variation in C and P mineralizing enzyme activities and 50% of N mineralizing enzyme activities beneath *E. nigrum* ssp. *hermaphroditum* were explained by variations in soil total C, pH, and NH_4^+−N between elevations. Those same factors explained <53% of the variation in C and nutrient mineralizing enzyme activities in soils beneath *B. nana* (Fig. 3). These results suggest that elevation effects on C and nutrient mineralizing enzymes beneath *E. nigrum* ssp. *hermaphroditum* are more strongly derived from the alteration of soil physiochemical properties than they are for *B. nana*. At this arctic site, soil total C and soil pH were more important than soil NH_4^+−N in influencing soil C and nutrient mineralization. Total soil C was the primary factor influencing C and N degrading enzyme activities, and soil pH was the primary factor influencing P mineralizing enzyme activity. As the energy source for microbial growth and substrate for C and N mineralization, soil organic C is correlated with hydrolytic enzyme activities (Sinsabaugh et al. 2008). Also, limited labile C availability can restrict microbial biomass growth in Arctic soils (Jonasson et al. 1999, Melle et al. 2015). Further, soil pH is a strong controller of extracellular enzyme activities directly and indirectly through its effects on microbial biomass and community composition, solubility, and degradability of soil organic C (Sinsabaugh et al. 2008, Eskelinen et al. 2009, Leifeld et al. 2013, Hendershot et al. 2017), and our results thus reinforce that heterogeneity in soil pH should be taken into account when studying responses of soil processes to climate change in tundra areas.

Conclusions

We observed contrasting effects of sites differing in elevation on C and N transformations in soils beneath two common, but functionally contrasting, arctic shrub species. A lower and warmer elevation site was associated with elevated C-degrading enzyme activities in the humus layer beneath the deciduous ectomycorrhizal shrub *Betula nana*, but these processes were reduced at the lower and warmer elevation site in humus beneath the evergreen ericoid mycorrhizal shrub *Empetrum nigrum* ssp. *hermaphroditum*. Moreover, we found that soil C, N, and P transformations were tightly coupled beneath *E. nigrum* ssp. *hermaphroditum*, but not beneath *B. nana*. This study provides novel information about the species-level consequences of elevation-associated long-term warming impacts on belowground C, N, and P transformations across our study system, and highlights the strong species-specific impacts and their interactions with environmental changes on belowground biochemistry in the Arctic.

Acknowledgments

The Carlsberg Foundation, Formas and Göran Gustafssons Foundation for Environmental Research in Lapland supported this work and The Danish National Research Foundation supported the Center for Macroecology, Evolution and Climate. The National Natural Science Foundation of China (Nos. 41373087), and the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Terrestrial Ecosystem Sciences Program (Award Number DE-SC0010562) also supported this work.

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